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Ciliate distribution amongst soil aggregates

BY

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Synopsis: A new technique for distinguishing between inter and intra-aggregate populations of soil ciliates is described using 5 minutes fumigation with chloroform and soil aggregates (∅ 2-5 mm.). Pore size distribution within the same size of aggregates was estimated by image analysis of back scattered electron scanning images.

Keywords: Soil ciliates, fumigation, soil aggregates, pore-size distribution.

INTRODUCTION

HATTORI (1988) suggested that the pore system of soil aggregates consists of two groups of pores; an outer region with pore necks wider than 2.5 to 6 µm in diameter and an inner region with smaller pore necks. The evidence to support this distribution was based principally on the results of his serial washing and sonication technique. Using this technique, he found most of the prorozoa were easily washed off the aggregates and suggested that these protozoa resided either on the aggregate outer surfaces or in pores larger than 5 µm diameter in the outer region of the aggregates. He believed the inner region of the aggregate was colonised mainly by bacteria. Previously, VARGAS and HATTORI (1986) had found that when *Colpoda* sp. and *Aerobacter aerogenes* were inoculated on to sterile soil aggregates, the ciliates were mostly recovered in the serial washings and were only rarely recovered after sonication. As these authors had found that sonication did not affect the viability of *Colpoda* sp., HATTORI interpreted their results as supporting his hypothesis. This paper reports preliminary attempts to measure the porosity of

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soil aggregates using scanning electron microscopy and image analysis as well as providing extra experimental evidence to test HATTORI's hypothesis.

I. – MATERIALS AND METHOD

A) Soil

The sandy loam soil (pH 5.5, 5.2% C) used in these trials was collected from Dunecht, Aberdeenshire (Countesswells Soil Association & Series, S layer, Glentworth and Muir 1963) and passed successively through a series of sieves (Endecotts, London, U.K.) with holes of decreasing diameter (10, 5, 2, 1, 0.5, 0.25, 0.125 and 0.063 mm). Approximately 200 mg of the aggregates from each size range were used in the first trial to determine whether grinding the aggregates in a pestle and mortar destroyed the protozoan population. Each sample of aggregates was incubated in 10 ml of sterile soil extract for 7 days at 15°C. Two replicates of each treatment were used. In the subsequent fumigation trials, wet soil aggregates (∅ 2-5 mm) were used. Five randomly chosen aggregates in 10 ml sterile soil extract were used in each replicate and they were incubated for 7 days at 15°C. Three replicates were used in all treatments except one where five replicates were used. The moisture contents of the wet soil aggregates were determined after drying in an oven set at 105°C for 24 hours.

B) Fumigation

Wet sieved soil aggregates on watch glasses were fumigated with chloroform vapour inside sealed glass Petri dishes. Some cotton wool soaked with $CHCl_3$ was placed below the watch glasses to saturate the atmosphere inside the sealed petri dishes 5 minutes before use.

C) Protozoan population estimates

Three grammes of soil as wet sieved aggregates (2.56 g dry wt) were fumigated with chloroform for 5 minutes and then suspended in 50 ml of Tris buffer (50 mM, pH 7.6) for the extraction procedure described by GRIFFITHS and RITZ (1988) with four replicate samples being examined under the fluorescence microscope. The aggregates were shaken (wrist action shaker SD 100, Gallenkamp, London, U.K.) at setting 7 for 10 minutes after fumigation to disperse the soil in the buffer suspension. Fluorescent cells were regarded as metabolically active ciliates, if the cells were 10 µm long, stained with 2-(4-iodophenyl)-3-(-4-nitrophenyl)-5-phenyltetrazolium chloride (INT) and were ovoid in shape. An aliquot of the same Tris buffer suspension was used to prepare a three-fold dilution series with soil extract for most probable number (MPN) estimates as described by DARBYSHIRE *et al.* (1974).

D) Aggregate porosity estimates

Twenty randomly chosen wet sieved soil aggregates (∅ 2 to 5 mm) were air dried and embedded in LR white acrylic resin (London Resin Co., Basingstoke, U.K.) under reduced pressure. After polymerisation, the aggregates in the resin blocks were cut approximately in half and then into thin slices (approx. 2 mm thick). Thin slices from the centres of the aggregates were mounted on stubs, polished to 1 µm finish, coated with 20 nm of carbon, examined in a scanning electron microscope (Cambridge Stereoscan S4, Cambridge, U.K.) and the backscattered electron scanning images (BESI) recorded (DARBYSHIRE *et al.*, 1985). The porosity of montages of contact prints (M × 200) of complete cross sections of nine aggregates were estimated using an image analyser (System III, Analytical Measuring Systems, Cambridge, U.K.). One montage was prepared from each of the nine aggregates. The montages were analysed in a step-wise manner with fields of view of 4,240 μm^2. When a field of view overlapped the edge of the aggregate, the area outside the aggregate was delineated by the image editor and not included in the analysis. The total porosity and the distribution pattern of pores in different size classes were determined using suitable software supplied by the manufacturers of the analyser and an Apple II microcomputer (Apple Computer, Cupertino, California, U.S.A.). The inner and outer regions of the aggregates were distinguished as follows. A grid system of lines was superimposed on each aggregate with the area of each square being 4,240 μm². Any area of the aggregate that fell in the outer squares was regarded as within the outer region of the aggregate. The remaining areas of the aggregates were regarded as the internal regions of the aggregates.

II. — RESULTS

A) Effect of grinding aggregates on protozoan survival

Representative samples of wet sieved soil aggregates with diameters ranging from 5 to 0.63 mm were incubated in liquid soil extract for 7 days at 15°C and examined for the presence of flagellates, amoebae and ciliates. Half the samples of aggregates were ground to a paste with a sterile pestle and mortar before the incubation. The results are summarised in Table I and show that protozoa were not eliminated either by grinding the aggregates or by wet sieving.

TAB. I. — **Protozoa associated with soil aggregates of different diameters (mm) after wet sieving and grinding in a pestle and mortar.** (F = Flagellates, A = amoebae, C = ciliates, Control = unsieved soil.) Two replicates in each treatment.

	Range of diameters (mm)						
	Control	5-2	2-1	1-.5	.5-.25	.25-.125	.125-.063
Ground aggregates	FCA	FC	FC	FCA	FCA	FCA	FCA
Unground aggregates	FCA	FC	FCA	FCA	FCA	FCA	FCA

B) Effect of chloroform fumigation for different periods of time

Wet sieved soil aggregates (∅ 2-5 mm, H_2O content 17-20%) were exposed to $CHCl_3$ vapour for a range of periods of time. Half the aggregates were ground in a pestle and mortar before fumigation. The results for ciliates are summarised in

TAB. II. — **Number of cultures with ciliates associated with soil aggregates (∅ 2-5 mm) after wet sieving, with and without grinding in a pestle and mortar before fumigation with $CHCl_3$.** Three replicates used in all treatments except in treatment * where five replicates were used.

	Fumigation time (min.)									
	0	0	0	5	5	5*	10	15	15	20
Ground aggregates	3	3	3	0	0	0	0	0	0	0
Unground aggregates	3	3	3	3	3	4	0	2	0	0

Table II. Five minutes fumigation destroyed the ciliate population in the ground aggregates, but some ciliates developed amongst unground aggregates after 5 minutes fumigation when examined after 6 days at 15°C. Few ciliates appeared amongst aggregates ground or unground when the fumigation period was extended to 10 minutes or more. It is suggested that the greater resistance of ciliates to $CHCl_3$ when associated with unground aggregates is because some of the ciliates were inside the aggregates and contact with $CHCl_3$ was delayed. Flagellates proved to be far more resistant to $CHCl_3$ than ciliates and could be recovered from unground aggregates after 20 minutes fumigation.

C) Estimates of ciliate populations inside soil aggregates

Using wet sieved soil aggregates (∅ 2-5 mm) fumigated with $CHCl_3$ for 5 minutes, four population estimates of metabolically active ciliates associated with the aggregates were 121, 168, 116 and 76 g^{-1} dry wt of soil respectively. Using the MPN method the estimates were below 781 ciliates g^{-1} dry wt of soil.

D) Aggregate porosity

The porosity in nine aggregates (∅ 2-5 mm) are displayed in Figures 1, 2 and 3 and the heterogenous nature of the aggregates as mixtures of organic and mineral fragments are shown in low power (BESI) cross-sectional views of four of the aggregates (*Fig.* 4). For example, aggregate 2 has a large vertical void, aggregate 4 has a large unidentified fragment in the centre and aggregate 9 consists mainly of a central core of a rock fragment. If one assumes that pores of 2.5 and 6 μm diameter are spherical in outline, then the equivalent areas will be 19.63 and 28.27 μm^2 respectively. It can be seen from Figures 1, 2 and 3 that both inner and outer regions of the aggregates contain many pores above and below that size range and the data do not support HATTORI's (1988) suggestion that soil aggregates consist of an inner region with smaller pores and an outer region with larger pores. Nevertheless, apart from the pore distribution in aggregates 2 and 9, there is a slightly larger frequency of pores in the outer region of the aggregates compared with the inner region in most of the size classes. DARBYSHIRE (1976) found that the soil ciliate, *Colpoda steini*, did not multiply in small cultures in the laboratory, when the soil samples were placed on sand bath tension tables and exposed to tensions with pF values in excess of 2. Such a critical suction corresponds to an effective pore diameter of 30 μm at 15°C. If one assumes that all pores of 30 μm diameter are spherical in outline, the corresponding area is 706.86 μm^2. It can be seen from Figures 1, 2 and 3 that all the nine aggregates contain some pores in excess of 706 μm^2 and large enough to accommodate some of the common soil ciliates.

III. – DISCUSSION

The results of this preliminary investigation suggest that small numbers of ciliates can exist inside aggregates of 2-5 mm diameter. It remains to be determined whether these intra-aggregate ciliates were encysted from the time of aggregate

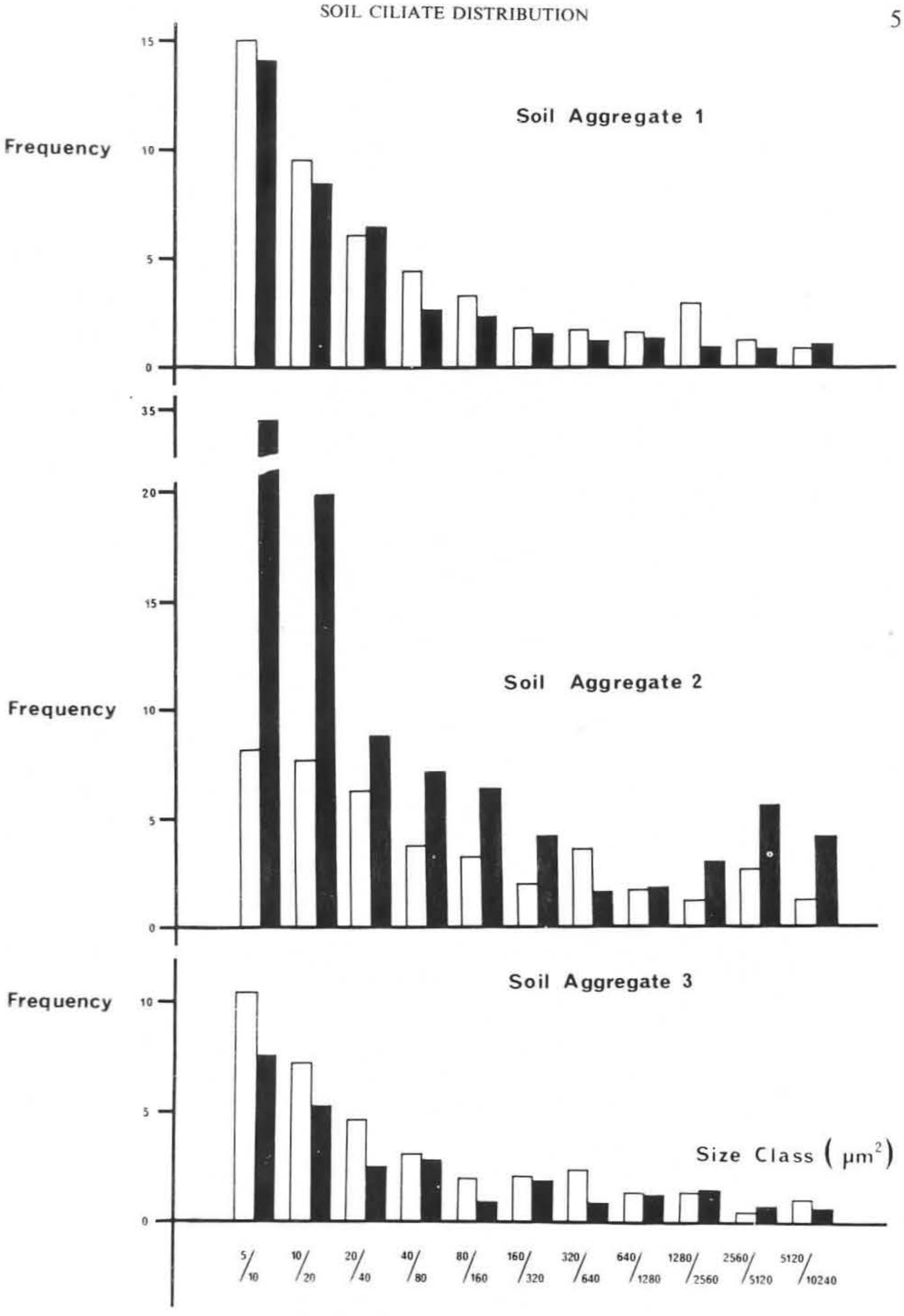

FIG. 1. – Pore size distribution in soil aggregates (Ø 2-5 mm) Nos. 1, 2 and 3. The open columns represent the outer region of the aggregate and the black columns represent the inner region.

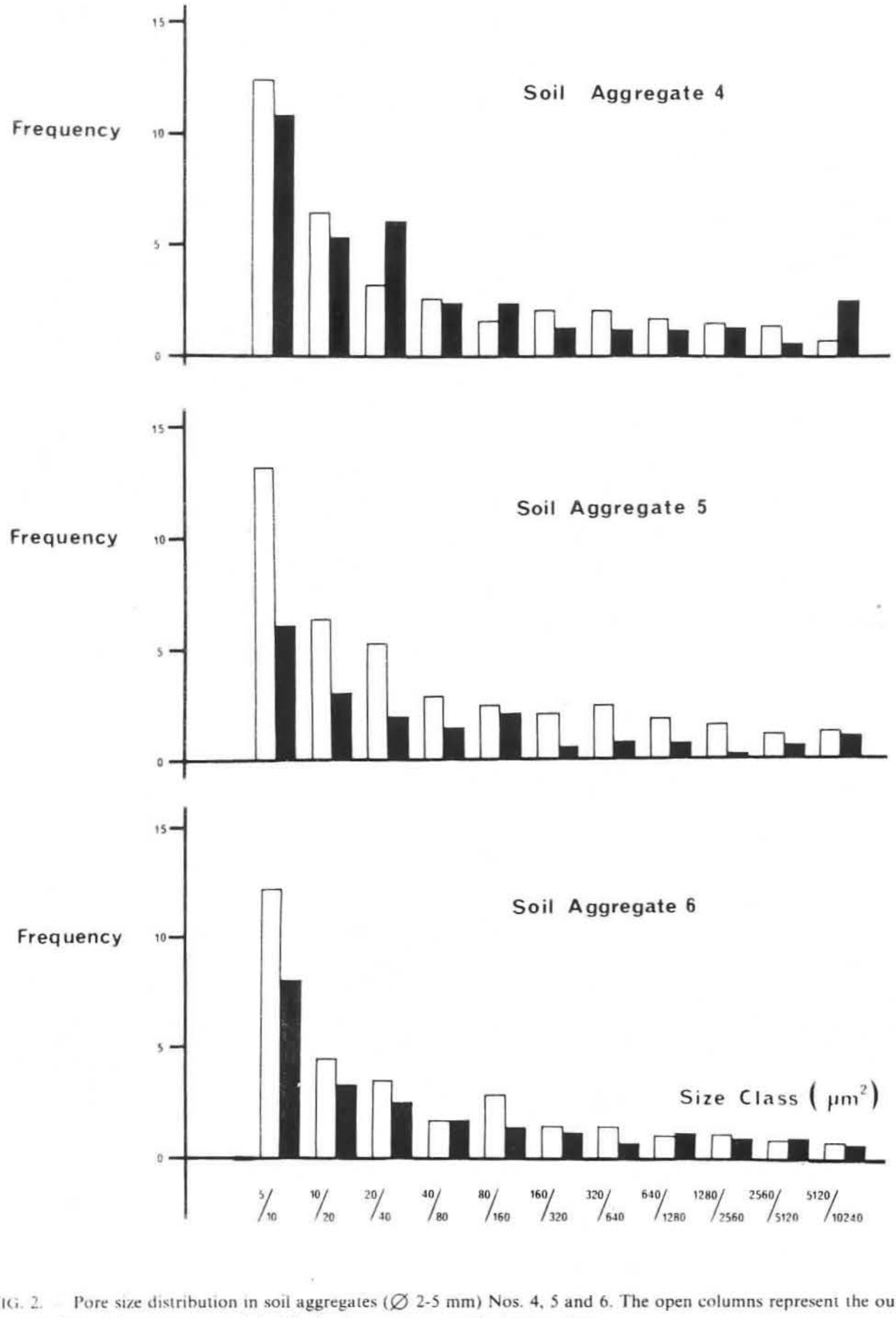

FIG. 2. Pore size distribution in soil aggregates (∅ 2-5 mm) Nos. 4, 5 and 6. The open columns represent the outer region of the aggregate and the black columns represent the inner region.

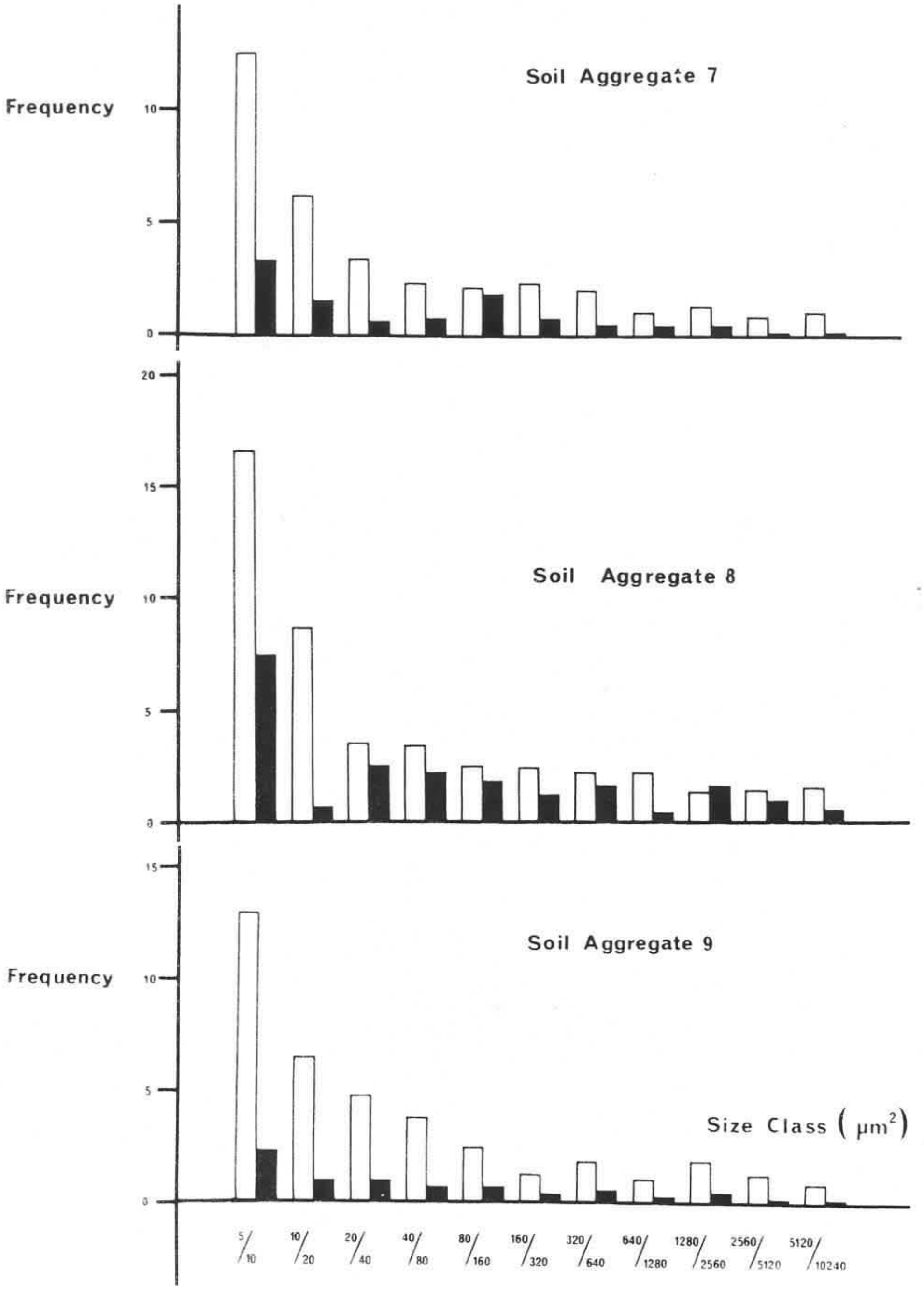

FIG. 3 — Pores size distribution in soil aggregates (∅ 2-5 mm) Nos. 7, 8 and 9. The open columns represent the outer region of the aggregate and the black columns represent the inner region.

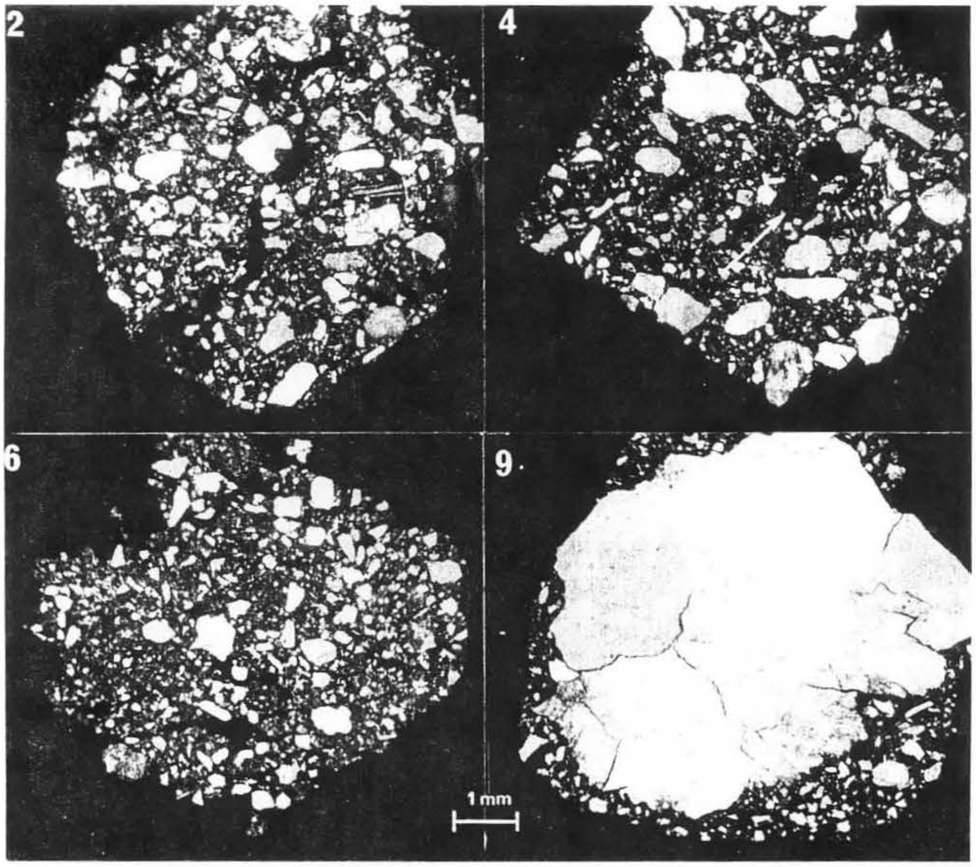

FIG. 4. — Low power cross sectional view (BESI) of soil aggregates (∅ 2-5 mm) Nos. 2, 4, 6 and 9. Arrow marks unidentified fragment in aggregate 4.

formation or whether there was a period of trophozoite activity. As these population estimates are below many published estimates of soil ciliate populations, it is concluded that the majority of the ciliate population in the soil resides in the inter-aggregate pore space. Further studies are required to confirm this possibility or whether ciliates are more abundant in aggregates of another size. The porosity data show that the outer region of aggregates of 2-5 mm diameter do not consistently possess larger pores than the inner region of the aggregate, as suggested by HATTORI (1988). Since sonication of trophozoites and cysts of *Colpoda steini* in soil suspensions has been found in this laboratory to destroy all ciliate cells in 8 minutes (SNEDDON, 1983), HATTORI's hypothesis that the absence of ciliates after sonication indicates there are no ciliates in the central part of soil aggregates should be treated with some caution until more data are available. Nevertheless, the porosity data in the present study show that some pores exist inside the aggregates that could accommodate some soil ciliates. Many serial sections are required to establish the degree of connectivity between the soil pores. The small number of ciliates found after fumigation of uncrushed aggregates, suggest that many of the microfloral cells inside aggregates are protected from ciliate predation until the aggregate is at least partly disrupted. Kilbertus (1980) also showed with transmission electron microscopy that small soil aggregates of 35 μm diameter contained several discrete microbial microcolonies in apparently blind pores. It is probable that many of these cells would be protected from ciliate predation. The relative resistance of flagellates to $CHCl_3$ fumigation in the present study may be because some of these protozoa inhabit the smaller pores in the aggregates and less accessible to $CHCl_3$.

Summary

A new technique for distinguishing between inter and intra-aggregate populations of soil ciliates is described. Using short periods of fumigation (5 minutes) with chloroform, small populations of ciliates were recovered apparently from inside uncrushed soil aggregates (∅ 2-5 μm). Soil flagellates were more resistant to such fumigation and some of these protozoa may survive in smaller pores than the ciliates. Pore size distribution within the aggregates was estimated by image analysis of backscattered electron scanning images.

Résumé

Distribution de ciliés dans les agrégats du sol

Une nouvelle technique permettant de distinguer les populations de ciliés vivant dans ou à la surface des agrégats du sol a été décrite. Après une courte fumigation au chloroforme (5 minutes), on a pu récupérer quelques petites populations de ciliés provenant probablement de l'intérieur d'agrégats de sol non écrasé (∅ 2-5 mm). Les flagellés du sol sont plus résistants à une telle fumigation car capables de vivre dans des pores de diamètre inférieur à ceux où sont les ciliés. Une approche de la distribution du volume poral est réalisée par des coupes d'agrégats observées au microscope électronique à balayage et analysées par un analyseur d'image.

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